REMARKS/ARGUMENTS

Claims 1-3, 10, and 12-19 remain pending in this application, and are rejected. Claims 4-9, 11, and 20-39 have been withdrawn from consideration. New Claims 40-50 have been added.

A. Restriction

In paragraph 2 of the Final Office Action, the Examiner indicated that Claims 1-19 were drawn to a method of folding a polypeptide and that Claims 20-39 were drawn to a method of screening for an optimal folding environment for a denatured peptide. Applicant acknowledges the Examiner's restriction requirement and has withdrawn the second set of claims from Examination.

B. Drawings

In accordance with the Examiner's instructions in paragraph 4 of the Final Office Action, formal drawings will be filed once the application is allowed.

C. Claim Rejections Under Section 102

1. Gorovits Reference

In paragraphs 7-8 of the Final Office Action, the Examiner rejected Claims 1-3, 10 and 15-16 as being anticipated by the Gorovits Article (1997). Applicant respectfully traverses the rejection in light of the amended claims.

a. The claimed folding method requires addition of the osmolyte to the already-formed polypeptide-chaperonin complex to promote folding greater than that which is achieved by using chaperonins or osmolytes alone.

As background, and as set forth in the attached Declaration under Section 1.132, it is important to understand that protein folding is defined as the process whereby the hydrophobic parts of a flexible polymer become buried inside the interior as the flexible polymer collapses to a more organized and defined three dimensional structure. Protein folding is therefore a complex

reaction that can choose multiple routes at the same time. Thus, correct protein folding involves a balance between these multiple folding reactions (generically called pathways) that lead to correct and functional proteins (enzymes, structural proteins etc.) vs. those routes that lead to off-pathway incorrectly folded proteins. In many cases, where proteins often misfold, it is because the off-pathway routes are faster than the routes that lead to correctly folded proteins.

The present invention is directed to a novel method for folding a denatured polypeptide which involves two steps. First, the claims require that the polypeptide be bound to the chaperonin to form a chaperonin-complex. Second, the claims require that the "chaperonin-polypeptide complex [be exposed] to an osmolyte, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide." Since the already-formed chaperonin-polypeptide complex is exposed to the osmolyte, the chaperonin must first be added to the polypeptide to form a complex. Then, the osmolyte is added to the solution. The advantages of this two-step process are set forth in Applicant's specification:

> [T]he two-step folding procedure provides several important and unexpected benefits. The procedure combines the chaperonin's ability to prevent aggregation and even unfold the misfolded intermediates with the inherent structural stabilization and enhancement of folding afforded through the use of osmolytes. As the experiments with GSΔ468 demonstrate in Table 1, this combination can produce a remarkable synergistic amplification of protein folding in vitro. Because the refolding of denatured protein is performed in two steps; the solution parameters such as temperature, ionic strength, and protein concentration can be adjusted independently to insure both the efficient chaperonin-substrate complex formation and the optimal substrate release and refolding in the presence of osmolytes. The high stability of the complex allows for an easy manipulation of solution conditions without the significant loss of the folding proteins due to aberrant aggregation at higher concentrations. In the case of GSΔ468, substrate concentration was initially kept low in order to avoid rapid aggregate formation and insure high chaperonin-to-substrate stoichiometry. Once the complex is formed, however, the substrate concentration can be increased to enhance the concentration-dependent second order GSΔ468 assembly reaction as shown in Table 2.

See Paragraph [0063] (emphasis added). Applicant has further amended independent Claim 1 to clarify the synergistic advantages of the present invention: namely, that the two step process results in "promoting [that] is greater than that which is achieved using chaperonins and osmolytes alone."

2. The Gorovits Article does not teach or suggest Applicant's two-step procedure.

In the Gorovits Article, urea was used to denature the protein itself (equivalent to step (a) of Applicant's claimed invention) -- not to promote the folding of the polypeptide as required by the claimed invention. That is, the urea solubilize the hydrophobic interior of proteins and was used to unfold proteins. Next, after denaturing/unfolding the protein, the Gorovits Article indicates that the unfolded protein was diluted with buffer and the chaperonin so that the urea concentration did not exceed 0.5M. Clearly, the urea (the purported osmolyte) was not added to the protein after the addition of the chaperonin. Because the Gorovits Article does not teach or suggest addition of the osmolyte to the already-formed chaperonin-polypeptide complex, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

In contrast to the prior art, the precise order of this chaperonin/osmolyte protein folding process of the present invention is crucial to insure successful screening for optimal conditions. The folding protein is initially captured by the oligomeric chaperonin to form an arrested chaperonin-protein substrate complex. Applicant has repeatedly shown that this arrested form can hold to protein in a metastable but eventually foldable state for a long period of time (~2 hr at 37°C, Fisher, 1992). Once this complex is formed, the test osmolyte solution(s) can then be added to the arrested chaperonin-protein substrate complex and released into the test osmolyte solution where the ability of the osmolyte to influence successful protein folding can be evaluated. The order of addition is crucial because there are numerous instances where osmolyte

addition to a folding substrate alone prior to forming the arrested chaperonin-protein complex results in large scale protein misfolding (Voziyan et al., 2000; Voziyan and Fisher, 2000, attached to November 24, 2003 IDS). For example, Figures 4A and 6 in the latter reference show the glutamine synthetase misfolds (no activity, increase in aggregation species) when TMAO alone (without the chaperonin) is added to refolding glutamine synthetase monomers. Thus, to specifically practice the Applicant's invention, it is necessary to first form the complex between protein (e.g. glutamine synthetase, malate dehydrogenase or rhodanese) and chaperonin (e.g. GroEL) and then add the test osmolyte (e.g. urea, glycerol or TMAO etc. see list in Table 1 patent specifications) and ATP to determine if added osmolyte will allow the protein to acquire its correct folded structure once it is released from the chaperonin.

In short, the Gorovits Article does not teach or suggest first forming a chaperonin-osmolyte complex and then "exposing [the] chaperonin-polypeptide complex to an osmolyte" as required by the claims. Thus, Applicant requests that the Examiner withdraw the rejection under Section 102.

3. The urea in the Gorovits Article does not facilitate or enhance folding as required by the claimed invention.

As discussed above, protein folding is a balance between pathways that lead to functional proteins and those that do not. In the present invention, urea essentially prevents or slows down this collapse (protein folding). Thus, although urea does not help the collapse of the folded protein to the correct structure, in the present invention, urea can slow down the other faster incorrect paths allowing for the slower correct folding routes to begin dominating. Urea therefore assists with protein folding in an indirect manner.

More specifically, in the present invention, low concentrations of urea (~1 M) enables one to control the speed of the misfolded pathways, which in turn prevents these misfolding

pathways from taking over and forcing all or most of the folding protein to go down the wrong path. Functionally, the high affinity chaperonin is able to bind partially folded proteins before the proteins get into trouble. Once the protein is released from the chaperonin, it still can choose between multiple paths for folding (misfolding vs. folding). When urea present, misfolding reactions down the wrong path (which is usually the faster collapse pathway) slows down when the protein is released from the chaperonin. If the protein does not fold to its proper correct (forms a protected oily interior) within a certain time frame, the protein will rebind to the chaperonin and it can repeat this process over and over again. Thus, the present invention involves a situation where slowing the misfolding protein down will enable more protein to fold to its native state. In short, urea actually helps the chaperonin folding platform capture and release the folding protein by preventing the off-pathway folding.

Evidence of this is shown in **FIG. 7** of the patent specification. In **FIG. 7**, folding is helped by urea because urea prevents the folding intermediate from rapidly collapsing to a misfolded form, allowing the chaperonin to bind it and efficiently fold it even when the folding protein is present in excess (more the 1 molecule folding protein per 1 chaperonin - the stoichiometry).

In contrast to the present invention, the Gorovits Article merely discusses the chaperonin interaction differences when one generates different denatured (misfolded or incorrectly folded) states of proteins (in this case, either completely unfolded proteins with urea or thermally denatured proteins (heat denaturation). Urea is not used as a folding aid for the protein (dihydrofolate reductase or DHFR) in question at all. Furthermore, the protein in question (DHFR) folds quite efficiently by itself without requiring the chaperonin. The urea that is present during the folding reaction is only there because the protein becomes an unfolded protein

in concentrated urea which is then diluted into the solution containing the chaperonin. The Gorovits Article simply does not teach or suggest the use urea to enhance the stoichiometry between the folding protein and the chaperonin.

More specifically, the Gorovits Article does not teach or suggest urea (the purported osmolyte) to promote folding as required by the claimed invention. The prior art shows that GroEL actually suppresses the folding of dihydrofolate reductase ("DHFR"). See Viitanen et al., "*Complex Interactions between the Chaperonin 60 Molecular Chaperone and Dihydrofolate Reductase*", Biochemistry, at pp 9716-9723 (1991) (cited in the Gorovits Article). GroEL only has the ability to release DHFR after ATP is added to the arrested DHFR-chaperonin complex. The released DHFR does not gain any advantage of folding yield with the chaperonin present because it does not require the chaperonin to fold unlike the particular protein substrate that was used (ΔV468 GS) in Applicant's patent application.

The Gorovits Article merely investigated whether differing denaturing conditions (urea vs. thermal) would change the chaperonin requirements (GroEL, GroES ATP vs. GroEL GroES ADP). The reference simply created a different denatured protein to bind to the chaperonin by using either heat or urea to denature the protein. The folding of the protein DHFR was initiated in the presence of GroEL with residual urea present. However, the inclusion of urea did not in any way ***promote*** the folding of DHFR to its native conformation as required by the claimed invention. See Claim 1 ("exposing said chaperonin-polypeptide complex to an osmolyte, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide"). Further, there is not any teaching or suggestion that the resulting "promot[ion] is greater than that which is achieved using chaperonins and osmolytes

alone". As such, for this additional reason, Applicant requests that the Examiner withdraw the rejection under Section 102.

2. Altamirano References

In paragraphs 9 to 11 of the Final Office Action, the Examiner rejected Claims 1-3, 10, 13-15 as being anticipated under 35 U.S.C. § 102 by Altamirano (1997) or Altamirano (1999) (together the Altamirano Articles). As discussed above, the claimed invention requires that the chaperonin-protein complex must be formed prior to the addition of the osmolyte. Because the Altamirano Articles do not teach or suggest such a step, Applicant respectfully traverses the rejection. The advantages to Applicant's two-step invention are discussed above, and need not be repeated here.

In the 1997 Altamirano Article, the mini-chaperone was mixed with refolding buffer (2M KCl and 2 M urea). Next, the denatured protein (cyclophilin A in 8M urea as a denaturing agent) is added to the suspension. As such, the chaperonin-protein complex is not formed prior to the addition of the osmolyte as required by the claims. In other words, the there is no teaching of suggestion of exposing the "chaperonin-polypeptide complex to an osmolyte, thereby promoting the folding of said polypeptide from its unfolded state to its folded state to yield a folded biologically active polypeptide." Because this limitation is not taught or suggested by the 1997 Altamirano Article, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

Likewise, in the 1999 Altamirano Article, the refolding buffer (containing potassium phosphate and arginine) is first mixed with the refolding gel (containing either (1) the binary matrix of DsbA and minichaperone or (2) the ternary matrix of DsbA, PPI, and minichaperone). The denatured scorpion toxin Cn5 is then added. See Fig. 2 (caption); Table 1 (caption). Again,

the chaperonin-protein complex is not first formed prior to the addition of the osmolyte as required by the claims. Because this limitation is not taught or suggested by the 1999 Altamirano Article, Applicant respectfully requests that the Examiner withdraw the rejection under Section 102.

Applicant has also added new independent Claim 40, which recites that the chaperonins of the present invention are "oligomeric". No new matter has been added by this amendment. See Paragraph [0065] of the specification (describing the oligomeric GroEL). In view of this Amendment, Applicant respectfully submits that the claimed invention is patentable over the cited references.

As discussed in Applicant's Amendment and Response of June 25, 2003, the claimed "oligomeric chaperonins" are not the same as the fragmented "minichaperone system" described in the cited Altamirano references. The minichaperone system is inferior to and will not function with most commonly used polypeptides. In this regard, the Wang et al., (1998) and Weber et al., (1998) articles included in the Information Disclosure Statement show that the Altamirano minichaperone system fails to fold various stringent chaperonin requiring protein substrates. In cases where successful folding with the minichaperone is observed, Altamirano and his coworkers modestly refolded one stringent class III chaperonin dependent protein, but only under conditions where rhodanese can fold by itself (so-called "permissive" 25° C folding conditions). See Wang et al., (1998) Figure 3A; Smith & Fisher (1995), Figure 1. Thus, the minichaperone fails to fold this protein to any degree under very controlled conditions (Wang et al., 1998, Figure 3 A and B in this figure - sht345 is the minichaperone fragment apical domain). Curiously, one of the helper proteins used in the Wang article (figure 3), namely casein, is a

general milk protein that is not even classified as a chaperone yet this protein does better at folding the test substrate than does the minichaperone fragment.

In addition, Wang et al., (1998) and Weber et al., (1998) showed that the minichaperone system could not function to fold malate dehydrogenase or the maltose binding protein. The minichaperone fails to fold its test substrate Rhodanese at 37°C (See Weber et al., (1998) Figure 3 B. In contrast, as illustrated in Example 8, the present chaperonin/osmolyte invention is capable of working with other substrates (such as malate dehydrogenase) See also (Tieman et al., 2001). Further, subsequent work by the inventors (Voziyan and Fisher) indicates that the present invention involving chaperonin/osmolyte systems shows that it works with citrate synthase, another one of the test substrates that the minichaperones will fail to fold. Furthermore, the present invention is able to fold various chaperonin substrates at much higher protein concentrations at physiological temperatures (37°C) than any of the in vitro methods available (Table 2) without excessive misfolding and aggregation (Fisher 1993 listed in patent specification). The primary difference between the claimed invention, which uses the oligomeric version of the chaperone, and that of the minichaperone fragment is that the latter cannot capture and hold folding polypeptides or stabilize a metastable protein state for any length of time.

In short, there is nothing in the prior art that teaches the use of an "oligomeric chaperonin"/osmolyte system to promote the folding of a polypeptide. Applicant respectfully submits that the Altamirano references do not teach or suggest the claimed invention. As such, withdrawal of the rejection is requested.

In view of the foregoing amendments and remarks, it is respectfully submitted that the claims are now in condition for allowance and eventual issuance. Such action is respectfully

requested. Should the Examiner have any further questions or comments in order to obtain allowance, he is invited to contact the undersigned attorney at the number listed below.

Acknowledgment of receipt is respectfully requested.

Respectfully submitted,

By: _____

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